

Campylobacter* susceptibility to ciprofloxacin and corresponding fluoroquinolone concentrations within the gastrointestinal tracts of chickens

M.B. Farnell¹, A.M. Donoghue¹, K. Cole², I. Reyes-Herrera², P.J. Blore² and D.J. Donoghue²

¹Poultry Production and Product Safety Research Unit, Agricultural Research Service, USDA, and ²Poultry Science Department, University of Arkansas, Fayetteville, AR, USA

2004/1329: received 17 November 2004, revised 31 May 2005 and accepted 1 June 2005

ABSTRACT

M.B. FARNELL, A.M. DONOGHUE, K. COLE, I. REYES-HERRERA, P.J. BLORE AND D.J. DONOGHUE. 2005.

Aims: This study evaluated the relationship between *Campylobacter* susceptibility and enteric fluoroquinolone concentrations in chickens treated with different doses of enrofloxacin.

Methods and Results: All chickens were challenged with seven fluoroquinolone sensitive *Campylobacter jejuni* (6.6×10^6 CFU per bird) at 2 weeks posthatch. At 26 days of age chickens were treated with 0 ($n = 29$ birds), 25 mg ml⁻¹ enrofloxacin (Baytril®, Bayer Corp., Shawnee Mission, KS, USA) for 3 days ($n = 45$ birds) or 50 mg ml⁻¹ enrofloxacin for 7 days ($n = 65$ birds) in the drinking water. The crop, upper ileum, lower ileum, ceca and colon contents were collected from both enrofloxacin treatment groups ($n = 5$ birds per day per treatment group) and nonmedicated controls. The minimum inhibitory concentration (MIC) of ciprofloxacin for *Campylobacter* increased for isolates from both treatment groups within the first day of dosing and the daily average ranged from 1.4 to 6.5 µg ml⁻¹ throughout the study. Although enteric fluoroquinolone concentrations were higher ($P < 0.05$) in birds dosed with 50 mg ml⁻¹ vs 25 mg ml⁻¹ enrofloxacin, there were no differences between the isolates collected from these groups for MIC values.

Conclusion: These data indicate, for the doses used, differences in gut fluoroquinolone concentrations do not produce isolates of *Campylobacter* with differing susceptibility to ciprofloxacin.

Significance and Impact of the Study: Using the manufacturers lowest, shortest duration dose vs the highest, longest duration dose of enrofloxacin did not change *Campylobacter* susceptibility to ciprofloxacin. However, ciprofloxacin MIC values for *Campylobacter* determined in this study were lower than previously reported.

Keywords: antibiotic resistance, broiler, *Campylobacter*, chicken, ciprofloxacin, enrofloxacin, fluoroquinolone, food-borne pathogen.

INTRODUCTION

The Centres for Disease Control (CDC) report that *Campylobacteriosis* is one of the leading causes of food-borne illness in the United States (CDC 2004). An infectious dose

as few as 500 organisms can cause gastroenteritis in humans (Fields and Swardlow 1999) by adhering to the gut epithelium and disrupting gut absorption. *Campylobacteriosis* is usually a self-limiting disease with symptoms lasting only 4–5 days, however, *Campylobacter* may be the aetiological agent of more chronic debilitating diseases such as Guillian–Barré syndrome and reactive arthritis (Ziprin 2004).

It has been estimated that up to 90% of all chickens carry *Campylobacter* and poultry is thought to be an important vector of the disease in humans (Stern and Line 1992). While *Campylobacter* is a zoonotic pathogen, it does not

*Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Correspondence to: Dan J. Donoghue, Department of Poultry Science, 0-408 Poultry Science Center, University of Arkansas, Fayetteville, AR 72701, USA (e-mail: ddonogh@uark.edu).

affect growth or development of the chicken (Stern *et al.* 1988; Jeurissen *et al.* 1998). It is unknown exactly how the bacteria is transmitted to the bird, but there are many sources of *Campylobacter* found in the environment such as insects, rodents and wild birds that may serve as carriers of the pathogen (Mead 1993).

Fluoroquinolones are used to treat diarrhoea in man (e.g. ciprofloxacin) and colibacillosis in poultry (e.g. enrofloxacin; McDermott *et al.* 2002; Ge *et al.* 2003). There have been recent concerns that the agricultural use of antibiotics may increase antibiotic resistance of food-borne pathogens (e.g. *Campylobacter*, *Salmonella*) and make medical treatment of these infections in humans more difficult (McDermott *et al.* 2002; Ge *et al.* 2003). While studies have shown that the treatment of colibacillosis with the fluoroquinolone, enrofloxacin, does not cause significant increases in resistant *Escherichia coli* (van Boven *et al.* 2003) there have been three recent reports that this treatment could select for ciprofloxacin-resistant *Campylobacter* in chickens (McDermott *et al.* 2002; Luo *et al.* 2003; Humphrey *et al.* 2005). However, these studies did not evaluate the relationship between enteric fluoroquinolone concentrations and resistance levels. This information could be important for the development of antibiotic dosing strategies to reduce drug-resistant *Campylobacter*. Therefore, the purpose of this study was to evaluate the dose response of the Food and Drug Administration (FDA) approved low and high dose of enrofloxacin and *Campylobacter* susceptibility within the gastrointestinal tract (crop, upper ileum, lower ileum, ceca or colon) in chickens.

MATERIALS AND METHODS

Experimental animals

Male broiler chickens were obtained on the day-of-hatch from a commercial hatchery and placed into three floor pens with supplemental heat. Chicks were provided water and a balanced, nonmedicated, maize-soybean ration *ad libitum* that met or exceeded the National Research Council guidelines for chicken nutrition (National Research Council 1994).

Experimental design

A total of 139 chickens were challenged with *Campylobacter jejuni* at 14 days posthatch and at 26 days of age treated with 0 ($n = 29$ birds), 25 mg ml⁻¹ enrofloxacin (Baytril®) for 3 days ($n = 45$ birds) or 50 mg ml⁻¹ enrofloxacin for 7 days ($n = 65$ birds) in the drinking water. Seven fluoroquinolone sensitive wild-type *Camp. jejuni*, collected from poultry carcasses, were individually sub cultured from glycerol stocks and passed twice (48 h per pass at 42°C) into seven

separate tubes containing *Campylobacter* enrichment broth (CEB, International Diagnostics Group, Bury, Lancashire, UK) and used for challenge. The seven strains were combined and all birds were orally gavaged with 6.6×10^6 CFU of *Camp. jejuni* per bird.

At 19 days of age (7 days prior to enrofloxacin dosing), cloacal swabs were collected to confirm *Campylobacter* colonization from two birds in each of the three treatment groups and these birds were maintained on the study. At 26 days of age, immediately prior to dosing (day 0), nine nonmedicated control birds (three from each treatment pen) were collected to evaluate *Campylobacter* colonization and their susceptibility to ciprofloxacin or fluoroquinolone content in all five-gut locations as described below. Following collection of these nine control birds, two treatment groups were dosed with enrofloxacin (Baytril®) as per the United States FDA approved manufacturer label directions. The low dose group ($n = 45$ birds) received 25 mg l⁻¹ of enrofloxacin in water for 3 days and the high dose group ($n = 65$ birds) received 50 mg l⁻¹ of enrofloxacin for 7 days. Intestinal contents from the crop, upper ileum, lower ileum, ceca and colon were collected from individual birds in both the low and high dose treatment group on each day of the 3 or 7 days dosing period and days 1–4, 7 and 14 postwithdrawal ($n = 5$ birds per day per group) for determination of both enteric antibiotic concentrations and the susceptibility of any *Campylobacter* isolated to ciprofloxacin (Bayer, Kansas City, MO, USA).

In addition to the nine control birds collected prior to dosing (day 0), 20 additional nonmedicated control birds were collected ($n = 2$ day⁻¹) during the same dosing and withdrawal period as birds receiving enrofloxacin. These control birds ($n = 2$ day⁻¹) were collected on day 1 (corresponds to the first day of enrofloxacin treatment), 3, 5, 7, 9, 10, 11, 14, 17 or 21 of the experiment. The control birds (total of 29 birds) were housed in the same building as enrofloxacin treated birds. A total of 695 samples were collected (five gut samples from each of 139 birds) and used for isolation of *Campylobacter* for minimum inhibitory concentration (MIC) and fluoroquinolone assays.

MIC assay

Antibiotic susceptibility of *Campylobacter* was quantitated using the agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2002). *Campylobacter jejuni* ATCC 33560 was used as a quality control organism and its ciprofloxacin MIC was 0.25 µg ml⁻¹, which is within the NCCLS recommended range (0.125–1.0 µg ml⁻¹, McDermott *et al.* 2002) for this organism. The agar dilution plates consisted of Mueller-Hinton agar (Difco, Sparks, MD, USA) with 5% defibrinated sheep blood. Fourteen plates were made with each one

receiving an increasing twofold (0.015–64 µg ml⁻¹) concentration of ciprofloxacin (ICN Biomedicals Inc., Aurora, OH, USA). Plates were used within 5 days of preparation as per the NCCLS recommendations (NCCLS 2002).

Intestinal contents were diluted 1 : 9 with CEB, vortexed and subcultured onto a Campy-Line Agar (CLA; Line 2001) plate for isolation. These samples were incubated for 48 h at 42°C in an atmosphere containing 5% O₂, 10% CO₂ and 85% N₂. Colonies of *Campylobacter* were periodically verified by the *Campylobacter* Latex Agglutination Test (Panbio Inc., Columbia, MD, USA) and the *Campylobacter* API (Biomérieux, Marcy, l'Etoile, France) biochemical test. Five colonies of *Campylobacter* was subcultured, from each individual sample, into 4.5 ml of CEB and incubated for 24 h at 42°C under anaerobic conditions. A 1 µl loop of the culture was then inoculated onto each agar dilution plate concentration and incubated at 37°C for 48 h. The plates were then observed for growth or no growth. The MIC was determined according to the NCCLS (2002) guidelines.

Antibiotic bioassay

Intestinal contents were diluted 1 : 3 with 1% phosphate buffer, pH 9.0, centrifuged at 1500 g for 20 min at 4°C, and the supernatant removed and stored at -80°C to be analysed later for enrofloxacin concentration using the method described by Donoghue and Schneider (2003). Briefly, *Klebsiella pneumoniae* (ATTC 10031, Manassas, VA, USA) was grown in tryptic soy broth (Difco) to a turbidity of 0.081 absorbance, which corresponds to approx. 9.2×10^8 *Kl. pneumoniae* per ml. The bacteria was diluted to a concentration of 1×10^6 CFU per ml and used to inoculate Mueller-Hinton agar (Difco) that had been previously cooled to 49°C in a water bath. Plates were made with the inoculated agar and six stainless steel penicylinders (Fisher Scientific, Pittsburgh, PA, USA) were evenly spaced onto each plate. On each plate three alternative penicylinders were filled with a standard (standards ranged from 25 to 5000 µg ml⁻¹) or unknown and the remaining cylinders were filled with a reference standard (400 µg ml⁻¹) that was used to correct for any plate-to-plate variation. The plates were then incubated overnight at 37°C and any resulting zones of inhibition were measured with an antibiotic zone reader (Fisher Scientific). A best-fit regression line using the diameter of the growth inhibition zones was calculated by the method of least-squares. The limit of sensitivity for this assay was 10 µg ml⁻¹ of enrofloxacin.

Statistical analysis

Data were analysed by analysis of variance using the Statistical Analysis System (SAS Institute 1994) general linear models program. Treatment means were partitioned

by least-squares mean (LSMEANS) analysis. Ciprofloxacin MIC values for *Campylobacter* were logarithmically transformed (log₁₀ CFU per ml) prior to analysis in order to achieve homogeneity of variance. The data in Fig. 1 are shown as arithmetic means for clarity of presentation. A probability of $P < 0.05$ was required for statistical significance.

RESULTS

Cloacal swabs collected from six control birds, 7 days prior to enrofloxacin dosing, were all positive for *Campylobacter*. The nine control birds (three from each treatment pen) collected immediately prior to the initiation of dosing (day 0) were all positive for *Campylobacter* in all five gut locations (Fig. 2). None of these samples produced any detectable fluoroquinolone activity (detection limit of 10 µg ml⁻¹).

During the enrofloxacin dosing and withdrawal period, the MIC of ciprofloxacin was similar for isolates from all five-gut locations. In addition, the concentration of fluoroquinolone was similar at all gut locations within either the low or high dose group ($P > 0.05$). Therefore, the enteric data were combined by dosing group (Fig. 1). Prior to dosing (day 0), all groups had isolates of *Campylobacter* that were inhibited by a low ciprofloxacin MIC (< 0.125 µg ml⁻¹). Approximately 24 h after dosing (day 1), the isolates from both the low and high treatment groups (average of five birds per day per treatment group) had higher average ciprofloxacin MIC values, which persisted throughout the treatment and withdrawal period (Fig. 1). However, there was no difference in the ciprofloxacin MIC value for *Campylobacter* ($P > 0.05$) isolated from the low or high enrofloxacin dose groups. The average ciprofloxacin MIC for *Campylobacter* (mean ± SEM) for the five birds collected each day was 3.0 ± 1.9 , 3.6 ± 1.8 , 6.0 ± 0.9 , 3.9 ± 0.8 , 5.7 ± 1.1 , 3.7 ± 0.5 , 3.8 ± 2.0 , 1.6 ± 0.7 or 6.5 ± 1.3 µg ml⁻¹ for the low dose group during the 3 days dosing period and 1–4, 7 or 14 days antibiotic withdrawal period respectively. For the high dose group, the average ciprofloxacin MIC for *Campylobacter* (mean ± SEM) for the five birds collected each day was 6.0 ± 2.0 , 1.4 ± 0.5 , 4.4 ± 1.1 , 5.6 ± 0.8 , 5.4 ± 1.0 , 5.2 ± 0.8 , 3.1 ± 0.6 , 5.9 ± 1.4 , 4.6 ± 0.6 , 3.9 ± 0.1 , 5.0 ± 0.8 , 5.6 ± 1.0 or 5.0 ± 1.0 µg ml⁻¹ during the 7 days dosing period and 1–4, 7 or 14 days withdrawal period respectively (Fig. 1). *Campylobacter* isolates collected from nonmedicated control birds had ciprofloxacin MIC values below 0.125 µg ml⁻¹ during the corresponding dosing and withdrawal period (Fig. 1).

Enteric fluoroquinolone concentrations were greater in the high vs low treatment group during the dosing and first day of antibiotic withdrawal ($P < 0.05$; Fig. 1). Fluoroquinolone activity was detected in the gut until 4 or 14 days postdrug

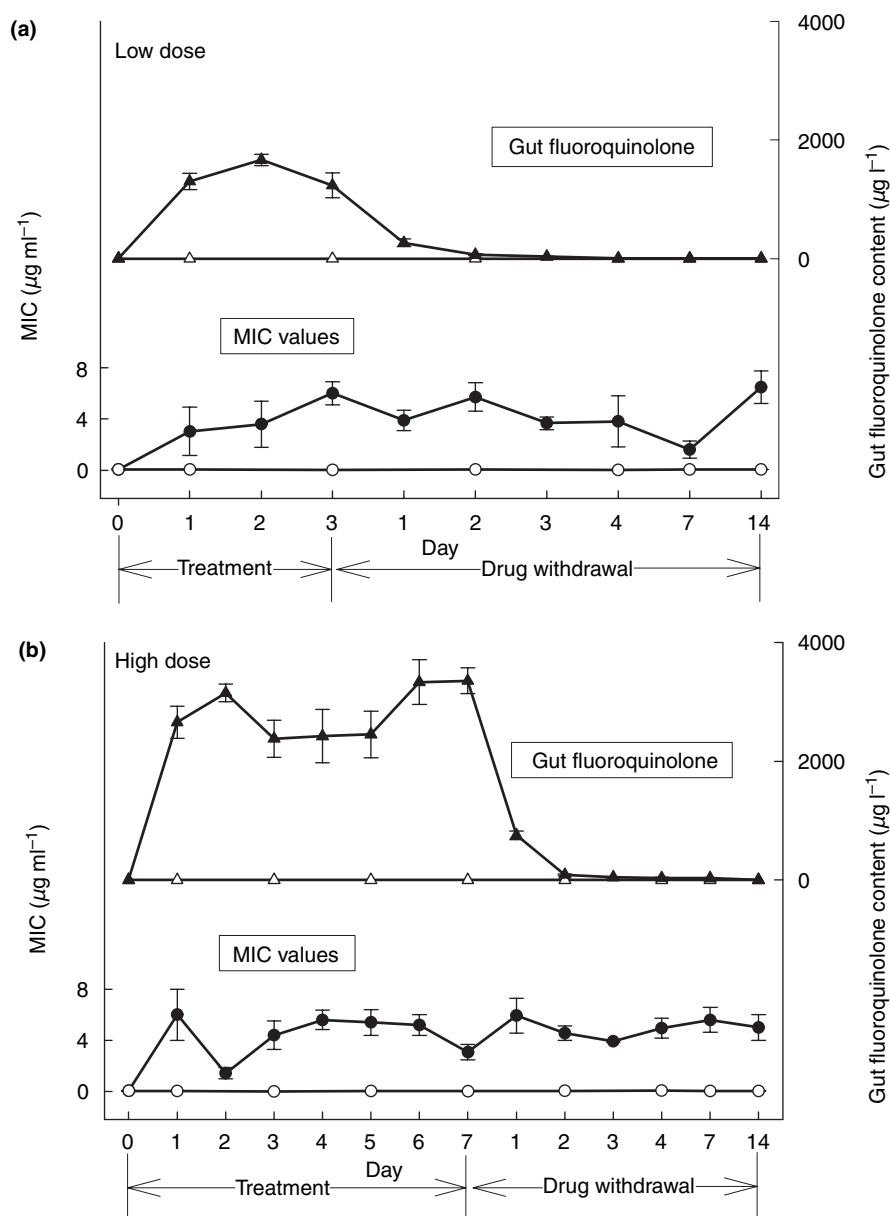
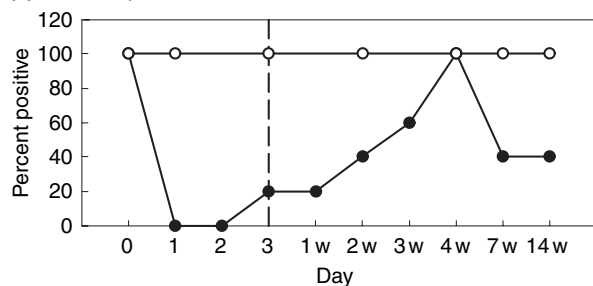
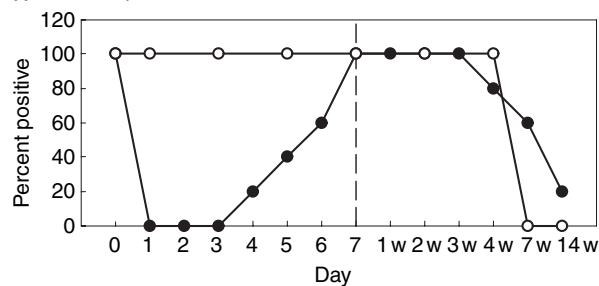
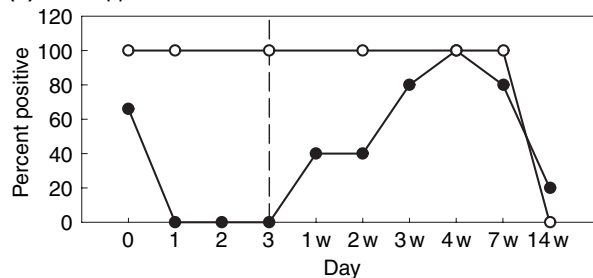
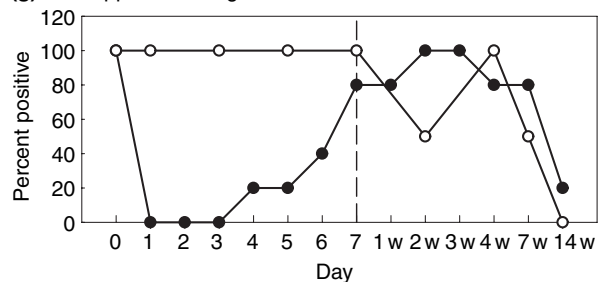
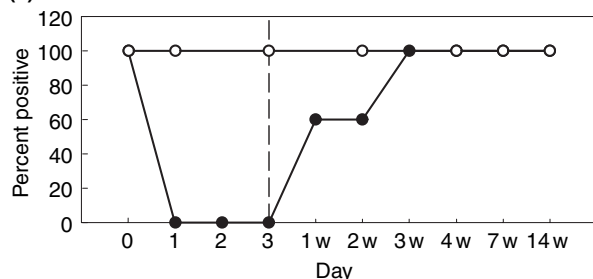
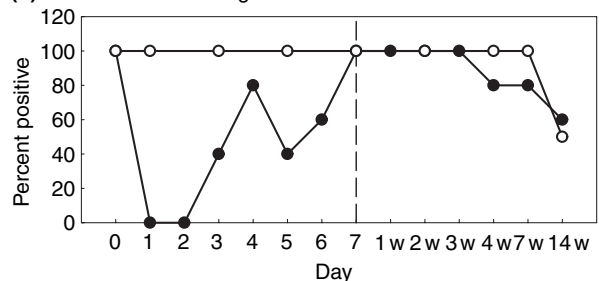
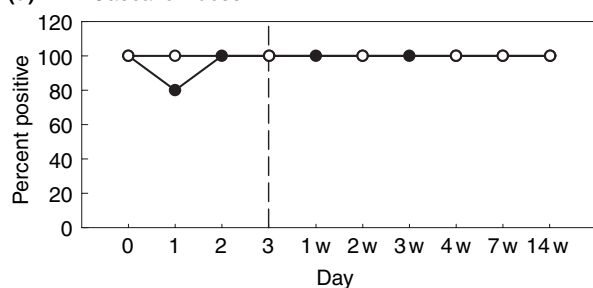
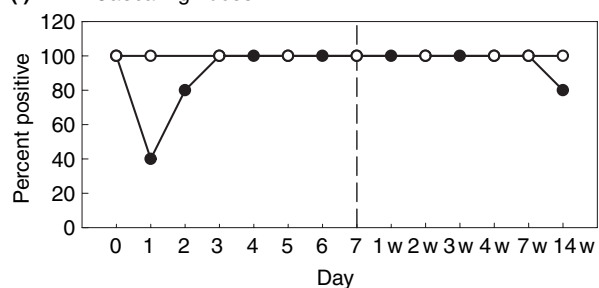
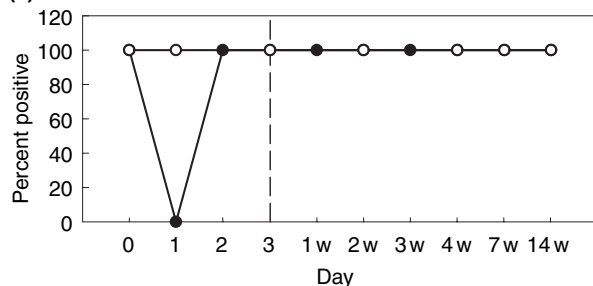
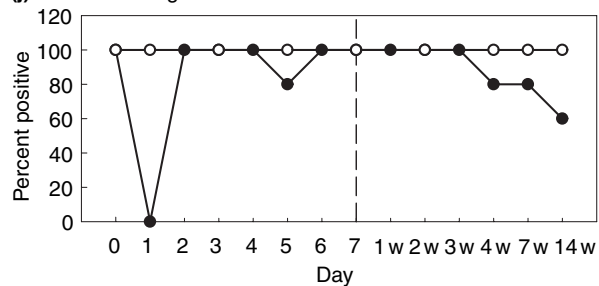


Fig. 1 Mean enteric fluoroquinolone concentrations and ciprofloxacin minimum inhibitory concentration (MIC) for *Campylobacter* in treated chickens. The (a) low dose treatment group received 25 mg l⁻¹ of enrofloxacin (Baytril®) for 3 days and the (b) high dose treatment group received 50 mg l⁻¹ of enrofloxacin for 7 days as per label directions. Samples were collected daily during the dosing periods (days 1–3 low dose treatment; days 1–7 high dose treatment) and days 1–4, 7 and 14 postdrug withdrawals. Gut contents were collected from the crop, upper ileum, lower ileum, ceca and colon of five chickens per enrofloxacin treatment per collection day for a combined total of 25 samples per collection day per treatment. Similarly, enteric contents were collected from nine control birds immediately prior to dosing (day 0) or two birds per day during the dosing and withdrawal period as indicated on the figure. There were no differences between the five gut locations for MIC values or fluoroquinolone concentrations in either the low or high dose group ($P > 0.05$) and, therefore, the enteric data were combined by dosing group. Mean gut fluoroquinolone concentrations (μg l⁻¹) are listed on the axis above the MIC (μg ml⁻¹) data. Control birds had no detectable fluoroquinolone (10 μg ml⁻¹). Open symbols indicate control groups; closed symbols indicate treatment groups

withdrawal for the low or high groups respectively. None of the control birds collected during the corresponding treatment or withdrawal period had any detectable fluoroquinolone.

After the first day of enrofloxacin dosing (day 1), *Campylobacter* was not detected (detection limit, 5×10^2 CFU per g of gut contents) in the crop, upper ileum, lower ileum or colon but was detected in the ceca for both the low

Fig. 2 Incidence of detectable *Campylobacter* within the crop, upper ileum, lower ileum, ceca and colon in treated chickens (○) control and (●) treated. The low dose treatment group received 25 mg l⁻¹ of enrofloxacin (Baytril®) for 3 days and the high dose treatment group received 50 mg l⁻¹ of enrofloxacin for 7 days as per label directions. Samples were collected during the dosing periods (days 1–3 low dose; days 1–7 high dose) and days 1–4, 7 and 14 postdrug withdrawals. Gut contents were collected from the crop, upper ileum, lower ileum, ceca and colon of five chickens per enrofloxacin treatment per collection day for a total of 25 samples per collection day per treatment. Similarly, enteric contents were collected from nine control birds immediately prior to dosing (day 0) or two birds per day during the dosing and withdrawal period as indicated on the figure. Percent *Campylobacter* positive incidence by location was calculated by dividing the number of *Campylobacter* positive samples by the total number of samples. The *Campylobacter* detection limit was 5×10^2 CFU per g of gut contents

(a) Crop low dose**(f)** Crop hi dose**(b)** Upper ileum low dose**(g)** Upper ileum high dose**(c)** Lower ileum low dose**(h)** Lower ileum high dose**(d)** Caeca low dose**(i)** Caeca high dose**(e)** Colon low dose**(j)** Colon high dose

and high dose groups (Fig. 2). On day 2 of dosing *Campylobacter* was detectable in both the ceca and colon in both treatment groups; by the end of the third day of dosing *Campylobacter* was detectable in the upper gastrointestinal tract in both treatment groups (Fig. 2). Nonmedicated control birds did not experience this reduction in *Campylobacter* incidence during either dosing period (Fig. 2).

DISCUSSION

Antibiotic usage in domestic food animals has created concerns about the development of antibiotic-resistant pathogens and transfer into the human population (van Boven *et al.* 2003). Although the regulatory withdrawal of antibiotics for use in animal production may reduce this problem, antibiotics are an important tool for the treatment of sick animals and their well being. Ideally, dosing strategies can be developed which will reduce the development of resistance and still allow the use of antibiotics to treat animal illnesses. One potential pharmacological approach is to alter the dosing amount or duration of antibiotic usage in an effort to either limit susceptibility changes or prevent resistance by killing the pathogen (Craig 1998).

Results from our study indicate that the ciprofloxacin MIC values for *Campylobacter* were not significantly different between birds dosed with the FDA approved lowest, shortest duration dose (25 mg l⁻¹ for 3 days) *vs* the highest, longest duration dose (50 mg l⁻¹ for 7 days; Fig. 1). This lack of difference occurred even though enteric fluoroquinolone concentrations were significantly higher in the birds receiving the highest, longest duration dose (Fig. 1). Although only using a single dosing period (5 days), Luo *et al.* (2003) also reported no differences in MIC values between birds dosed with either 25 or 50 mg l⁻¹ enrofloxacin. Thus, it appears that treating birds with the lower, shorter duration dose of enrofloxacin would not be an effective strategy to reduce ciprofloxacin susceptibility changes in *Campylobacter*.

An unexpected outcome of the current study was the determination that after the first day of dosing, both doses of enrofloxacin reduced *Campylobacter* below detectable limits (5×10^2 CFU per g of gut contents) in all five-gut locations in some birds, whereas others had detectable *Campylobacter* in only the ceca (Fig. 2). By the second day of dosing and afterwards, other areas of the gut started to recolonize with *Campylobacter*. These results support the observations by other researchers that only some of the birds remain colonized after enrofloxacin treatment (van Boven *et al.* 2003; Luo *et al.* 2003). Luo *et al.* (2003) speculated that these positive birds spread *Campylobacter* to pen mate chickens probably via horizontal transmission. Results from the present study support this idea and indicate that it may

only be the ceca that remains positive and acts as a reservoir for recolonization of the gastrointestinal tract and other pen mate chickens. It is also possible that *Campylobacter*, although undetectable, is still present within these areas of the gastrointestinal tract and repopulates the tract later in the dosing and withdrawal period.

It is unclear why the ceca remain positive for *Campylobacter* in some birds after enrofloxacin dosing. There were no differences in enrofloxacin concentrations between different gut locations within either dosing group ($P > 0.05$). The chicken ceca is an out pouching from the lower intestine and it is possible that differences in digesta circulation and/or physical properties within the ceca (Whittow 2000) may limit the interaction or efficacy of enrofloxacin on *Campylobacter*. In any event, if a concurrent therapy (e.g. prebiotics, bacteriocins) could eliminate cecal *Campylobacter*, then it may be possible to eliminate *Campylobacter* entirely and therefore the development of resistant *Campylobacter* in these dosed chickens.

Recent dosing studies with enrofloxacin have indicated an increase in ciprofloxacin MIC values for isolates of *Campylobacter* collected from medicated poultry (McDermott *et al.* 2002; Luo *et al.* 2003; Humphrey *et al.* 2005). To our knowledge, the breakpoint for ciprofloxacin resistance for *Campylobacter* has not been established. As described by McDermott *et al.* (2004), the British Society for Antimicrobial Chemotherapy (BSAC) and the Comité de L'Antibiogramme de la Société Française de Microbiologie (SFM) propose a resistant breakpoint of 4 µg ml⁻¹ for ciprofloxacin. These authors indicate these *Campylobacter* resistance breakpoints are based largely on the population distribution of MIC values, but lack clinical efficacy data (McDermott *et al.* 2004). Both McDermott *et al.* (2002) and Luo *et al.* (2003) reported MIC values much higher (32 µg ml⁻¹) than this proposed resistance breakpoint. Results from the current study indicate much lower MIC values than reported for these two studies. Most of our mean daily MIC values ($n = 5$ birds per day per dosing group) were either slightly below or above the proposed resistance breakpoint (results section and Fig. 1). These averages ranged from 1.4 to 6.5 µg ml⁻¹ throughout the dosing and withdrawal period. It is difficult to compare our results with Humphrey *et al.* (2005) because these authors used a lower resistance breakpoint of 1 µg ml⁻¹ for ciprofloxacin and only determined if isolates were above or below the breakpoint ciprofloxacin MIC value of 1 µg ml⁻¹.

The differences in MIC values between the current study and those of McDermott *et al.* (2002) and Luo *et al.* (2003) may be explained by different techniques, experimental design or variable response of different isolates when exposed to enrofloxacin. The FDA authors (McDermott *et al.* 2002) evaluated *Campylobacter* in pooled fecal samples collected from the litter. It is possible the dynamics of

Campylobacter resistance in fecal samples exposed to environmental conditions is different than within the bird's gastrointestinal tract. Enteric samples may be a better indicator of *Campylobacter* susceptibility to antibiotics and potential consumer exposure since the majority of *Campylobacter* contamination on edible carcasses is primarily due to contamination from enteric contents and not external fecal contamination on feathers (Berndtson *et al.* 1992; Musgrove *et al.* 2001). Another important issue is that McDermott *et al.* (2002) conclusions were based on analysis of pooled samples and pooling samples may bias experimental results (Silleby 2003). These authors determined ciprofloxacin MIC values for *Campylobacter* from five sets of five pooled samples. If only one of the five samples in each pool had high MIC values, then that entire pool would test out with high values.

Luo *et al.* (2003) also dosed chickens with enrofloxacin and reported that the majority of ciprofloxacin MIC for *Campylobacter* was $32 \mu\text{g ml}^{-1}$ with some samples in the $8\text{--}16 \mu\text{g ml}^{-1}$ range. These authors analysed cloacal swabs from individual chickens using the Etest for MIC determination. Although this was a well-designed study, recently, Ge *et al.* (2002) compared the Etest to the agar dilution method and 'the Etest tended to yield much higher resistant MICs than those measured by agar dilution at the resistant end of the MIC ranges'. According to their data (Ge *et al.* 2002), multiple *Campylobacter* isolates collected from retail meat samples having ciprofloxacin MIC $>4 \mu\text{g ml}^{-1}$ by agar dilution were determined to be $>32 \mu\text{g ml}^{-1}$ by Etest. Thus, the higher MIC obtained by Luo *et al.* (2003) may be due to method differences.

Another possible explanation for the differences in MIC values observed between the current and previous studies is that diverse *Campylobacter* isolates respond differently to enrofloxacin challenge. This idea is supported by the two companion papers of Griggs *et al.* (2005). When fluoroquinolones were used to dose multiple poultry flocks containing wild-type *Campylobacter* isolates, these authors observed various isolates having different MIC values. Furthermore, unlike the results from the present and previous studies (McDermott *et al.* 2002; Luo *et al.* 2003), they reported a significant decline in resistant strains in the weeks after treatment compared to the dosing period (Humphrey *et al.* 2005). They also observed a significantly lower percentage of resistant *Campylobacter* in one flock during the dosing period. Thus, some of the differences in MIC values observed in the present study *vs* previous studies may be due to the variability of the isolates' response to enrofloxacin treatment.

In conclusion, using the FDA approved enrofloxacin low dose, short duration or high dose, long duration in chickens did not change *Campylobacter* susceptibility to ciprofloxacin. It would appear, for the doses used, this is not an effective

strategy to reduce changes in *Campylobacter* susceptibility to ciprofloxacin. Alternative approaches need to be developed, such as concurrent therapies (e.g. prebiotics, bacteriocins) in an effort to reduce or eliminate susceptibility changes in *Campylobacter* during enrofloxacin treatment.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Michael F. Slavik for providing the *Camp. jejuni* isolates, Dr Ronald W. McNew for his expertise with statistics and data analysis, Ms Kinnari Pandya for her help with media preparation and necropsies and Mr M. Wally McDonner for his expertise as facility manager.

REFERENCES

- Berndtson, E., Tivemo, M. and Engvall, A. (1992) Distribution and numbers of *Campylobacter* in newly slaughtered broiler chickens and hens. *Int J Food Microbiol* **15**, 45–50.
- van Boven, M., Veldman, K.T., de Jong, M.C.M. and Mevius, D.J. (2003) Rapid selection of quinolone resistance in *Campylobacter jejuni* but not in *Escherichia coli* in individually housed broilers. *J Antimicrob Chemother* **52**, 719–723.
- CDC (2004) Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food selected sites, United States, 2003. *Morb Mortal Wkly Rep* **53**, 338–343.
- Craig, W.A. (1998) Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* **26**, 1–12.
- Donoghue, D.J. and Schneider, M.J. (2003) Comparison between a bioassay and liquid chromatography-fluorescence-mass spectrometry for the determination of incurred enrofloxacin in whole eggs. *J AOAC Int* **86**, 669–674.
- Fields, P.I. and Swerdlow, D.L. (1999) *Campylobacter jejuni*. *Clin Lab Med* **19**, 489–504.
- Ge, B., Bodeis, S., Walker, R.D., White, D.G., Zhao, S., McDermott, P.F. and Meng, J. (2002) Comparison of the Etest and agar dilution for *in vitro* antimicrobial susceptibility testing of *Campylobacter*. *J Antimicrob Chemother* **50**, 487–494.
- Ge, B., White, D.G., McDermott, P.F., Girard, W., Zhao, S., Hubert, S. and Meng, J. (2003) Antimicrobial-resistant *Campylobacter* species from retail raw meats. *Appl Environ Microbiol* **69**, 3005–3007.
- Griggs, D.J., Johnson, M.M., Frost, J.A., Humphrey, T., Jørgensen, F. and Piddock, L.J.V. (2005) Incidence and mechanism of ciprofloxacin resistance in *Campylobacter* spp. isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone treatment. *Antimicrob Agents Chemother* **49**, 699–707.
- Humphrey, T.J., Jørgensen, F., Frost, J.A., Wadda, H., Domingue, G., Elviss, N.C., Griggs, D.J. and Piddock, L.J.V. (2005) Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrob Agents Chemother* **49**, 690–698.
- Jeurissen, S.H.M., Janse, E.M., van Rooijen, N. and Claasen, E. (1998) Inadequate anti-polysaccharide responses in the chicken. *Immunobiology* **198**, 385–395.

- Line, J.E. (2001) Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. *J Food Prot* **64**, 1711–1715.
- Luo, N., Sahin, O., Lin, J., Michel, L.O. and Zhang, Q. (2003) *In vivo* selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. *Antimicrob Agents Chemother* **47**, 390–394.
- McDermott, P.F., Bodeis, S.M., English, L.L., White, D.G., Walker, R.D., Zhao, S., Simjee, S. and Wagner, D.D. (2002) Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *J Infect Dis* **185**, 837–840.
- McDermott, P.F., Bodeis, S.M., Aarestrup, F.M., Brown, S., Traczewski, M., Fedorka-Cray, P., Wallace, M., Critchley, I. *et al.* (2004) Development of a standardized susceptibility test for *Campylobacter* with quality control ranges for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem. *Microb Drug Resist* **10**, 124–131.
- Mead, G.C. (1993) Problems of producing safe poultry: discussion paper. *J R Soc Med* **86**, 39–42.
- Musgrove, M.T., Berrang, M.E., Byrd, J.A., Stern, N.J. and Cox, N.A. (2001) Detection of *Campylobacter* spp. in ceca and crops with and without enrichment. *Poult Sci* **80**, 825–828.
- National Research Council (1994) *Nutrient Requirements of Poultry*, 9th edn. Washington, DC: National Academy Press.
- NCCLS (2002) *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard M31-A2*, 2nd edn. Wayne, PA: National Committee for Clinical Laboratory Standards.
- SAS Institute (1994) *SAS Users Guide*. Cary, NC: SAS Institute.
- Silley, P. (2003) *Campylobacter* and fluoroquinolones: a bias data set? *Environ Microbiol* **5**, 219–230.
- Stern, N.J. and Line, J.E. (1992) Comparison of three methods for recovery of *Campylobacter* spp. from broiler carcasses. *J Food Prot* **55**, 663–666.
- Stern, N.J., Bailey, J.S., Blankenship, L.C., Cox, N.A. and McHan, F. (1988) Colonization characteristics of *Campylobacter jejuni* in chick ceca. *Avian Dis* **32**, 330–334.
- Whittow, G.C. (ed.) (2000) *Sturkie's Avian Physiology*, 5th edn. San Diego, CA: Academic Press.
- Ziprin, R.L. (2004) *Campylobacter* and campylobacteriosis: what we wish we knew. In *Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions* ed. Beier, R.C., Pillai, S.D., Phillips, T.D., Ziprin, R.L. pp. 73–86. Ames, IA: Blackwell Publishing and the Institute of Food Technologists.